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ENZYMATIC INTERESTERIFICATION OF CANOLA OIL AND HEAR STEARINE

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EXECUTIVE SUMMARY

Abstract

The objective of this research was to investigate the application of some new technology in order to produce an all Canola based margarine stock. The new technology involved the use of an enzyme (lipase) to alter the physical properties of Canola products. The enzyme lipase rearranged the chemical structure of a combination of Canola oil and fully saturated rape seed oil. The product resulting from the rearrangement had physical properties such as melting point, softening point and solid fat index that should interest margarine manufactures. The reaction took place at 60°C and the product contained considerably fewer trans fatty acids than does margarine stock currently made from partially hydrogenated Canola oil. The economical implications of this process are yet to be studied.

Summary

The main objective of this research was to investigate the possibility of using lipase to interesterify Canola oil and high eurcic acid rapeseed oil (HEAR) stearine with the resulting product having physical properties that might be useful in margarine manufacture. Lipase was found to catalyse the interesterification between Canola oil and other sources of lipids including free fatty acids, HEAR stearine and other triglycerides. Proof that the reaction has taken place can be found in the GLC and HPLC evidence presented in the full technical report.

Enzymatic interesterification of Canola/HEAR Stearine blends does cause a noted change in the physical properties of the blends and the resulting products appear to have potential as all Canola based margarine stocks. For example, the melting points for the 10 and 20% HEAR stearine blends drop from

>50°C to below 35°C which is close to body temperature, resulting in the "melt in the mouth" character required for margarines. It appears that between 20 and 25% HEAR stearine would be the best base to start with. The softening point, cloud point, and solid fat index (SFI) data varify these conclusions.

The physical properties of the enzymatically interesterified samples do not appear to be very different from similar blends that have been chemically interesterified. Advantages of the enzymatic interesterification are the lower temperature and less stringent moisture requirements for the reaction. The disadvantage of the enzymatic interesterification may be the unknown economical implications of the reaction, but that would have to be determined by an oil processing company.

Two types of enzymes were used and the NOVO enzyme (Lipozyme ™) appears to be best suited as it is currently commercially available. Other biotechnology firms are also now supplying lipase for the reaction and the most economical source should be searched out by any company wishing to employ this new technology. It should be noted that patents on this technology do exist (e.g. Unilever Ltd in England), and these should be examined prior to using these techniques.

Abstract

The objective of this research was to investigate the application of some new technology in order to produce an all Canola based margarine stock. The new technology involved the use of an enzyme (lipase) to alter the physical properties of Canola products. The enzyme lipase rearranged the chemical structure of a combination of Canola oil and fully saturated rape seed oil. The product resulting from the rearrangement had physical properties such as melting point, softening point and solid fat index that should interest margarine manufactures. The reaction took place at 60^{o}C and the product contained considerably fewer trans fatty acids than does margarine stock currently made from partially hydrogenated Canola oil. The economical implications of this process are yet to be studied.

BACKGROUND AND OBJECTIVES

Canola oil is hydrogenated in order to change the physical properties of the oil with the ultimate goal of utilizing the hydrogenated products in margarines and shortening products. During hydrogenation the formation of some transfatty acids and positional isomers occurs and this has led to some concern in regard to the nutritional quality of the hydrogenated oil.

An alternative to hydrogenation is interesterification of the canola oil with a fully hydrogenated oil such as palm, sunflover or canola stearine. Interesterification is currently accomplished by the use of a highly reactive chemical catalyst under very dry conditions at elevated temperatures.

The interesterification of Canola oil with the enzyme lipase has been investigated in our laboratory under a DSS contract that terminated November 85. Patents covering this technology exist but this technology has not been applied to Canola products. Lipase has been utilized to interesterify Canola oil with lauric acid or trilaurin and it appears that Canola oil has also been interesterified with HEAR stearine. The interesterification by positional analysis of the rearranged triglycerides has been confirmed. The advantages of enzyme catalyzed interesterification are that the reaction can take place at room temperature (or higher for HEAR stearine) and there is no requirement for toxic chemical catalysts. Several lipases have positional specificity that can produce products not possible with the random chemical interesterification. The enzyme can be attached to a carrier, recovered after the reaction, and utilized repeatly.

The main objective of this project was to develop a method for use of an enzyme instead of a chemical process to interesterify canola oil with a fully

hydrogenated oil such as oil from palm, sunflower or Canola stearine. The resultant product would be changed physically so that it could be used in place of hydrogenated Canola oil for margarine and shortening. The process could yield a nutritionally superior product to the present margarines made from hydrogenated Canola oil since no transfatty acids or positional isoners would be present. The process could also be carried out at room temperature.

The specific objectives of this research were to: (a) investigate the reaction parameters such as enzyme source, time of reaction, temperature and moisture content that would result in the desired new triglycerides without the production of excessive monoglycerides or diglycerides or free fatty acids; (b) investigate the crystal forming properties and soild fat indexes of the interesterified oils; (c) perform long term storage stability on the new products to evaluate oxidative and crystal stability.

EXPERIMENTAL METHODS

Materials: Canola oil and fully hydrogenated high erucic acid rape seed oil (HEAR stearine) were donated by CSP Foods Ltd, Altona, Manitoba. They were stored at -20°C. Lauric acid, stearic acid, triacylglycerols and fatty acid methyl ester standards were purchased from Sigma Chemical Co., St. Louis, Mo. U.S.A. Solvents and other routine chemicals were obtained from BDH Chemicals.

Enzymes: Two types of lipases were used in the present study. One was porcine pancreatic lipase purchased from Sigma Chemical Co. and the other was a microbial lipase obtained from NOVO Industries (Denmark) marketed under the trade name "lipozyme™". Both these enzymes were specific for the hydrolysis of fatty acids from 1 and 3 positions of triacylglycerols. The porcine pancreatic lipase as obtained from the manufacturer displayed little or no interesterification activity although it has good hydrolytic activity. To maximize and stabilize the interesterification activity, it was necessary to hydrate the enzyme, adsorb it on a suitable support material such as diatomaceous earth and dry under vacuum (75 - 100 mm Hg) at room temperature (23-25°C). The enzyme thus prepared had reasonably good interesterification activity. The NOVO "lipozyme" was derived from Mucor meihei and had been immobilized on an anion exchange resin which had an average particle size of 300 um. This enzyme preparation needed no further treatment and had a high interesterification activity.

Enzymatic interesterification: Fifty grams of a mixture of Canola oil and HEAR stearine, Canola oil and lauric acid, or Canola oil and stearic acid were transferred to a 100 ml jacketted Wheaton bottle. The bottle was connected to a circulating water bath and the mixture melted by raising the temperature of

the water bath to 80°C. The melted mixture was cooled to 60°C and held at this temperature during the course of the reaction. Sufficient amounts of lipase were added to the mixture and stirred continuously with a magnetic stirrer. The head space of the reaction vessel was flushed with nitrogen and the bottle closed tightly. The rection was allowed to proceed for a predetermined length of time. The time required for the reaction to reach an equilibrium state varied with the amount of enzyme and the total amount of moisture in the reaction system. When a large quantity of the product was required, the reaction was carried out with 250 g of Canola oil-HEAR stearine mixture in a 500 ml Wheaton bottle. After the reaction, the stirring was stopped and the enzyme settled down. The interesterified product was separated from the enzyme by filtering through a double layer of "KIMWIPE" tissue and stored at 20°C in screw capped jars, after flushing the headspace with nitrogen.

Chemical interesterification: Mixtures of Canola oil and HEAR stearine in various proportions were prepared and dried by heating at 150°C for 5 min. The mixture was cooled to 80°C and 50 grams of this mixture was transferred to a 100 ml jacketted Wheaton bottle. The bottle was connected to a circulating water bath which was maintained at 80°C. The catalyst used in the chemical interesterification was sodium-potassium alloy (1:3). This alloy was prepared in the laboratory by melting on a hot plate under liquid paraffin, 3 parts of potassium metal with 1 part of sodium (both from British Drug House). On melting, the metals fused together and became a homogeneous mass. The alloy thus prepared was stored under liquid paraffin in a desiccator. Before use, a small amount of the alloy was transferred to a beaker containing n-hexane and the liquid parqffin rinsed off. Usually 0.1 to 0.15 g of this alloy was added per 50 g Canola-HEAR stearine mixture. The mixture containing the catalyst was

stirred by a magnetic stirrer. After an initial lag period of 15 to 25 minutes, the color of the reaction mixture turned brown indicating the production of the "active" catalyst. After 2 hours, by which time the reaction had reached an equilibrium state, the reaction mixture was transferred to a separatory funnel and 100 ml hot water (65°C) was added, while the head space was being continuously flushed with nitrogen. After thoroughly mixing, the oil layer was allowed to separate. The oil layer was removed and washed again 3 times with 100 ml portions of water, extracted once with 100 ml of cold 95% ethanol, and finally dried by heating at 120°C for 10 min. The interesterified product was stored in screv capped jars at -20°C.

Gas Liquid Chromatography: The fatty acid composition of various samples were determined by preparing the methyl esters of the samples and analysing them to gas liquid chromtography. The methyl esters were prepared according to the method described by Hitchcock and Hammond (1978), with some minor modifications. About 75 to 80 mg of the fat sample was weighed into test tubes with teflon lined screv caps (100 mm x 10 mm), and 1 ml of heptadecanoic acid dissolved in hexane (10.88 mg/ml) was added as the internal standard. Hexane was evaporated off by heating the tubes under nitrogen on a heating block (80°C). The tubes were cooled to room temperature, and 2 ml of methanolysis reagent (sulphuric acid:methanol:toluene 1:20:10 v/v/v) was added and capped tightly. The reaction was carried out by heating the tubes at 100°C for 30 min on a heating block. After the reaction, the tubes were cooled to room temperature and 2 ml distilled vater was added followed by 2 ml n-hexane. The contents of the tubes were thoroughly mixed and the organic layer alloved to separate. The organic layer was removed by a Pasteur pipette and dried over anhydrous sodium sulphate (2 g). One ul of this was injected directly on the

column for the quantitative determination of fatty acids.

The methyl esters were analysed by a Hewlett Packard 5750 gas chromatograph equipped with dual columns and flame ionization detectors. The results were integrated and recorded by a Hewlett Packard integrator. The columns (6' x 1/8") were packed with GP 10% SP2330 on chromosorb WAW (Supelco Canada Ltd., Oakville, Ont.). Helium, at a flow rate of 30 ml per minute, was used as the carrier gas. Injection port and detectors were maintained at 250°C and 240°C respectively. The initial temperature of the oven was 150°C and it was programmed to rise at the rate of 8°C per min to a maximum of 235°C.

Thin Layer Chromatography: Thin layer chromatography (TLC) was used to separate triacylglycerols from other reaction products like free fatty acids, monoacylglycerols and diacylglycerols. Samples of 50 mg oil in chloroform were applied to precoated aelivated sylica gel-60 plates (0.25 mm) using a Camag chromatogharger. The plates were developed using a solvent system of hexane:ether:acetic acid (50:50:1). The bands were visualized by exposing to iodine vapour.

High Performance Liquid Chromatography: The triacylglycerol profiles of various interesterified and uninteresterified samples were determined by reverse phase high performance liquid chromatography (RP-HPLC). A Beckman Model 342 liquid chromatograph (Beckman Instruments, Inc., Berkeley, California) equipped with a 20 ul injection loop, a Micro-Guard ODS-5S (4.6 x 40 mm) pre-column (Bio-Rad Laboratories, Richmond, California) and an LC-18 5 micron (4.6 x 250 mm) column (Supelco Canada, Oakville, Ont). An Altex model 156 refractive index monitor with flow through cells (Altex Scientific Inc., Berkeley, California) was used as the detector. Static mobile phase was used

in the reference cell. The sample and reference cells were maintained at 25°C by a constant temperature circulating water bath. The results were integrated and recorded by a Hewlett-Packard 3390A electronic integrator (Hewlett-packard, Avondale, Pennsylvania). A mobile phase consisting of acetonitrile and tetrahydrofuran (60:40 v/v) was delivered isocratically at a flov rate of 1.0 ml per minute. Samples were dissolved in chloroform (0.25 mg/ml) before injection. Triacylglycerol peaks were identified using authentic standards.

Viscosity Measurements: Measurements of viscosities of products at various temperatures were conducted using a Haake Rotovisco RV11 (Gebruder Haake, Berlin, W. Germany) consisting of a Haake FK water circulator, a Haake PG11 temperature programmer.

Cloud Points: Official AOCS method No. Cc 6-25.

Softening and Melting Points: Official AOCS method No. Cc 3-25, Cc 1-25.

Solid Fat Index: Official AOCS method No. Ccl 10-57.

RESULTS AND DISCUSSION

When Canola oil, without mixing with any other kind of oil or fat, was interesterified enzymatically there was no appreciable change in the triacylglycerol profile as determined by reverse phase high performance liquid chromatography (RP-HPLC). Except for the production of small amounts of diacylglycerols and the liberation of a molar equivalent amount of free fatty

acids, the composition of the interesterified product was not significantly different from the starting material. Even the melting point of Canola oil was not altered by interesterification with these enzymes. Usually the melting point of vegetable oil is expected to rise on randomization of its fatty acids. The lack of change of melting point and triacylglycerol composition seemed to indicate that distribution fatty acids in positions 1 and 3 of triacylglycerols of Canola oil was already random and interesterifying with 1,3 specific enzyme was without any effect.

The interesterification activity of these regiospecific enzymes could be demonstrated by reacting a mixture of Canola oil and a fatty acid and then analyzing the product for the incorporation of the added fatty acid into acylglycerols. Lauric acid, which is not a normal constituent of Canola oil, was chosen to be a reactant, and served as a convenient marker to follov its incorporation into acylglycerols. The reaction was carried out with stirring using a mixture of 95% Canola oil and 5% lauric acid and 2.5 g of porcine pancreatic lipase preparation (equivalent to 13,000 units of lipolytic activity, hydrated, adsorbed on diatomaceous earth and dried as described in the methods section) added per 50 g of oil-fatty acid mixture. After 72 hours, the interesterified product was separated from the enzyme by filtration and the extent of incorporation of lauric acid into triacylglycerol determined by combined thin layer chromatography (TLC) and HPLC. It was found that 84% of the added lauric acid incorporated into triacylglycerol fraction. The HPLC profile of the interesterified sample clearly indicated the incorporation of lauric acid into triacylglycerols and concommitant disappearance of the free fatty acid (Fig. 1).

Preliminary studies had indicated that interesterification of oils by lipase was independent of the reaction temperature in the range of 20 to 65^{o}C

as long as the reactants were in the liquid state (Thomas et al. 1987). In such cases, the reactions were carried out at room temperature (25°C). Quite often, one of the reactants was solid at room temperature and it was necessary to use either a higher reaction temperature so that the reacting mixture was in a liquid state or use an inert organic solvent such as hexane or petroleum ether. Since the enzymes used in the present study were quite thermostable, the use of organic solvents was avoided, and the reactions were carried out at a higher temperature (60°C).

In another study to verify the incorporation of free fatty acids into triacylglycerols through enzymatic interesterification, 2 parts of Canola oil was mixed with 1 part of stearic acid and reacted with 3% NOVO "lipozyme" at 60°C. A similar reaction was carried out with Canola oil and lauric acid with the same enzyme under the same experimental conditions. After the reactions had reached an equilibrium state, the lipid fraction was separated and analyzed by RP-HPLC for the total triacylglycerol profile. On incorporation of stearic acid or lauric acid, the triacylglycerol profiles of Canola oil changed appreciably (Fig. 2). The interesterified samples contained not only a greater number of triacylglycerol peaks than the original Canola oil, but the retention times of these peaks had shifted. When stearic acid was interesterified with Canola oil, new triacylglycerols, which had retention times greater than any triacylglycerol in Canola oil, were formed. This is to be expected since incorporation of stearic acid raises the average molecular weight of triacylglycerols and therefore increases their retention times (the retention times of acylglycerols in HPLC analysis is proportional to equivalent carbon number where equivalent carbon number is equal to the number of carbon atoms in the molecule less twice the number of double binds: ECN=CN-2x double bonds). Incorporation of lauric acid, on the other hand, should lover the average

molecular weight of the triacylglycerols of Canola oil, and increase the proportion of low molecular weight triacylglycerol species. The HPLC profiles of acylglycerols presented in Fig. 2 confirmed this.

The extent of incorporation of the two fatty acids into the triacylglycerols of Canola oil was determined by fatty acid analysis. For this, triacylglycerols of the interesterified products were separated by TLC, their methyl esters prepared and the fatty acid composition determined by gas liquid chromatography (Table 1). This clearly shows that the added fatty acid is incorporated into triacylglycerols through interesterification.

Enzymatic interesterification usually results in the production of significant amounts of diacylglycerols and free fatty acids. The amount of diacylglycerol formed could be as high as 8%. The presence of diacylglycerol may have a beneficial or deleterious effect depending on the amount present in the product. From an industrial format of viev, controlling the amount of free fatty acids in a finished product is very important because free fatty acids lover the smoke point and are more susceptible to oxidative deterioration than acylglycerols.

The amount of free fatty acids produced during enzymatic interesterification, appears to be related to the amount of moisture present in the reaction system. Although water is not a reactant in the enzymatic interesterification, it is essential to maintain the catalytic activity of the enzyme (probably through maintaining proper protein conformation). A certain minimum amount of moisture must be present for the interesterification to occur and the rate of interesterification seemed to increase with increasing moisture content of the reaction system. With NOVO "lipozyme", maximum interesterification is reported to occur when the enzyme preparation contained 10% moisture and the enzyme was used at a 5% level. This level of moisture in

the reaction system also resulted in the production of a large amount of free fatty acids (up to 7%) and partial acylglycerols. To study the effect of moisture content of the reaction system on free fatty acid production, the NOVO enzyme which initially contained 7.98% moisture was carefully dried at room temperature (25°C) under vacuum (75-100 mm Hg) to varying levels of moisture. These partially dried enzyme preparations at a level of 5% were used to interesterify dried mixtures of Canola oil and HEAR stearine (60:40 w/w). Assuming that moisture content of the oil-stearine mixture was negligible, all of the moisture in the reaction system was derived from the enzyme preparation. All reactions were carried out at 60°C and completion of reaction required varied lengths of time, depending on the level of moisture. It is evident from Fig. 3 that free fatty acid production was proportional to the amount of moisture in the reaction mixture. By reducing the moisture in the reaction mixture it was possible to reduce the free fatty acid content of the final product. Reduction in the moisture levels, however, resulted in a slow rate of interesterification. A reaction mixture which contained 0.125% moisture took 48 h to produce a product which had the same HPLC profile as the control (0.4% moisture) which took less than 24 h to reach the equilibrium state (Fig. 4A and B).

In order to reduce the free fatty acid and diacylglycerol contents of the interesterified product it was necessary to reduce the moisture content of the NOVO "lipozyme". In all further experiments a partially dried "lipozyme" which contained only 2.5% moisture instead of 8% as supplied by the manufacturer was used. The free fatty acid production by this partially dried NOVO "lipozyme" was comparable to that produced by porcine pancreatic lipase which contained very lov moisture levels (about 0.05%) (Table 2). It is interesting to note that chemical interesterification produced very little free fatty acid. Some

amount of free fatty acids from chemically interesterified samples could be removed, however, by the alcohol extraction procedure in the final purification step. Another important observation is that the amount of free fatty acid produced was independent of the composition of the starting material.

Canola oil when plasticised by partial hydrogenation for the manufacture of margarine or confectionary fat, has a tendency to form large crystals of B type. In many food products this is an undesirable characteristic. Large B-type crystals result from relatively uniform fatty acid composition of Canola oil. Nearly 95% of fatty acids in Canola oil are C18 acids. This difficulty is usually overcome by blending Canola oil with oils or fats with dissimilar fatty acid composition. However the blended products do not have the same desirable physical properties as the interesterified product of the same composition as the folloving results would shov. In the present study fully hydrogenated high erucic acid rape seed oil (HEAR stearine) was used as the fat to blend with Canola oil. The blended mixture was then enzymatically or chemically interesterified. HEAR stearine was chosen because this fat had relatively high proportion of C22:0 acid which might produce the desired physical properties and smaller fat crystals in the product.

Fatty acid composition of HEAR stearine shoved that it contained only four saturated fatty acids (Table 3). The main fatty acid still was stearic acid (C18:0), but a third of the total fatty acid in HEAR stearine was behemic (C22:0). The blended mixture (60 parts of Canola oil and 40 parts of HEAR stearine) has a fatty acid composition which is quite in agreement with the fatty acid composition of individual components of the mixture.

Enzymatic interesterification using porcine pancreatic lipase: Pancreatic lipase hydrated and adsorbed on diatomaceous earth and then dried under

controlled conditions had reasonably good interesterification activity. Mixtures of Canola oil and HEAR stearine in various combinations were interesterified with this enzyme. It took nearly 72 hours at 60° for the reaction to come to equilibrium state with this enzyme. The reason for this slow rate of reaction may be related to lov moisture level of the enzyme (0.05%). A typical HPLC profile of acylglycerols of a mixture of Canola oil and HEAR stearine (60:40) and changes brought about on interesterification are shown in Fig. 4. The HPLC profile of the mixture has two distinct segments. In the first segment extending up 30 minutes in retention time, all peaks represent triacylglycerols of Canola oil while the peaks that emerged after 30 minutes were triacylglycerols of HEAR stearine. It is apparent that on interesterification some of the high melting triacylglycerols (higher retention time) of HEAR stearine disappeared, with the concommitant formation of lov melting triacylglycerols. Interesterification also resulted in the production of a greater number of nev species of triacylglycerols.

Enzymatic interesterification using NOVO "lipozyme": Although pancreatic lipase preparation could be used for interesterification, because of the long reaction time required and the poor reproducibility of interesterification activity from batch to batch, it was decided not to use this enzyme preparation in further studies. With NOVO "lipozyme" the reaction reached an equilibrium state within a relatively short time even when the moisture content of the enzyme was reduced from 8% to 2.5%. Therefore in all further studies enzymatic interesterification was done using this enzyme and the results were compared with chemically interesterified products.

Comparison between enzymatic and chemical methods of interesterification: The enzymes used in the present study are regiospecific. They catalyze the

interchange (exchange) of fatty acids within and among triacylglycerols from positions 1 and 3 only. In chemical methods on the other hand, all three positions of triacylglycerols are affected and this results in randomization of distribution of fatty acids. Mixtures of Canola oil and HEAR stearine were interesterified by both methods and the triacylglycerol composition determined by HPLC (Fig. 5). Although at first glance, the HPLC profiles of samples interesterified by both methods appears to be similar, there are considerable differences between the two. First, the retention times of similar looking peaks are not necessarily the same in both samples. This suggested that similar looking peaks may have different chemical (fatty acid) compositions. This observation can be verified only by isolating and determining the fatty acid composition of individual peaks. As could be expected from previous studies significant amounts of free fatty acids and diacylglycerols were formed during enzymatic interesterification. The amount of diacylglycerol produced during chemical interesterification was very small.

Effect of composition of the mixture of Canola oil and HEAR stearine on enzymatic interesterification: The overall HPLC profiles of interesterified samples were not affected by varying the proportion of HEAR stearine in the starting mixture (Fig. 6). However, with increasing amounts of HEAR stearine in the mixture more and more new species of high molecular weight (high retention time) triacylglycerol species were produced. For example, when the starting mixture contained 50% HEAR stearine, (Fig. 6), the interesterified product contained 24 peaks representing acylglycerols. However, when the HEAR stearine was reduced to 10%, the number of acylglycerol peaks in the interesterified product was only 15.

Physical properties of interesterified products: The effect of interesterification was readily apparent in the changed physical properties such as softening point, melting point, cloud point and viscosity. All these changes are brought about as a result of decrease in the solid fat content (or solid fat index = SFI) on interesterification.

a) Softening and melting points: Mixtures of Canola oil and HEAR stearine had relatively high softening and melting points even when the mixture contained as little as 10% HEAR stearine (Fig. 7 and 8). For example a mixture containing 40% HEAR stearine had a melting point of 62°C and lowering the percentage of HEAR in the mixture lowered the melting point only to 52°C. This is in contrast to the melting point of pure Canola oil which was found to be -6°C. Effects of interesterification on softening and melting points was quite dramatic. In the enzymatically interesterified sample there was a 34°C drop in melting point when the starting material cotnained 10% HEAR stearine and the drop in melting point was only 14°C when the HEAR stearine content was 40%. Apparently there were no notable difference between melting points of enzymatically and chemically interesterified samples.

b) Cloud points: The cloud points of mixtures and interesterified samples shoved the same general trend as the case of softening and melting points (Fig. 9). However, the cloud points were a fev degrees lover than the corresponding melting points. Once again there was no differences between chemically and enzymatically interesterified samples when the starting mixture contained 20% or more HEAR stearine. At lower levels of HEAR stearine in the starting mixture the cloud points appeared to be different between the two methods of interesterification.

(c) Viscosity: The viscosity of all samples (mixtures as well as interesterified samples) were very lov above 50°C, except when the mixture

contained very high levels of HEAR stearine (40%). This is to be expected since in these temperature ranges the samples were already in the liquid state. Below 50°C there was steady in the increase in the viscosities of mixtures with decreasing temperature. The temperature at which this steady increase started to occur seemed to be related to the percentage of HEAR stearine present in the mixture. On interesterification the viscosities of all samples decreased and there was no difference between viscosities of samples interesterified by chemical and enzymatic methods (Fig. 10). All interesterified samples had very lov viscosity at room temperature (25°C) where mixtures containing 40% and 30% HEAR stearine were solid at this temperature. Mixtures containing 20% and 10% HEAR stearine were semisolid at this temperature, and had significantly higher viscosities than their corresponding interesterified samples.

(d) Solid fat index (SFI): The SFI's of the mixtures were much different than the SFI's of the interesterified samples (Fig. 11). This was the expected result as the interesterifying procedure would be expected to lower the percent trisaturates, with a corresponding lowering of the solids content at any given temperature. It would appear that an interesterified product that originally had between 20 and 25% HEAR stearine would make a good margarine stock and should contain zero transfatty acids. The chemical and enzymatic interesterification resulted in products with very similar physical properties. Our laboratory is currently studying theoretical considerations for these similar physical properties.

Oxidative stability of interesterified samples: The interesterification process, especially the enzymatic method exposes the fat to relatively high temperature (60°-65°C) for a considerably long time (up to 72h). This coupled with the fact that enzymatic methods for interesterification results in the production of small amounts of free fatty acids suggested that oxidative

stability of interesterified product might be affected. This was studied by determining peroxide values of the samples during storage at room temperature (23-25°C) for 5 weeks. The results (Fig. 12) suggested that there was no apparent increase in the peroxide value of the uninteresterified sample (mixture) during storage. There was very moderate increase in the peroxide values of chemically intersterified samples. The initial peroxide value of chemically interesterified samples was lov because some of the peroxides were removed by the alcohol extraction procedure used in the final purification after chemical interesterification. The peroxide value of the enzymatically interesterified samples appeared to increase steadily over the period of storage although the maximum peroxide value observed by the end of 5 weeks was still well below the acceptable limits. It is probable that free fatty acids present in the enzymatically interesterified samples are the source of peroxides.

Summary

The main objective of this research was to investigate the possibility of using lipase to interesterify Canola oil and high eurcic acid rapeseed oil (HEAR) stearine with the resulting product having physical properties that might be useful in margarine manufacture. Lipase was found to catalyse the interesterification between Canola oil and other sources of lipids including free fatty acids, HEAR stearine and other triglycerides. Proof that the reaction has taken place can be found in the GLC and HPLC evidence presented in the full technical report.

Enzymatic interesterification of Canola/HEAR Stearine blends does cause a noted change in the physical properties of the blends and the resulting

products appear to have potential as all Canola based margarine stocks. For example, the melting points for the 10 and 20% HEAR stearine blends drop from >50°C to below 35°C which is close to body temperature, resulting in the "melt in the mouth" character required for margarines. It appears that between 20 and 25% HEAR stearine would be the best base to start with. The softening point, cloud point, and solid fat index (SFI) data varify these conclusions.

The physical properties of the enzymatically interesterified samples do not appear to be very different from similar blends that have been chemically interesterified. Advantages of the enzymatic interesterification are the lover temperature and less stringent moisture requirements for the reaction. The disadvantage of the enzymatic interesterification may be the unknown economical implications of the reaction, but that would have to be determined by an oil processing company.

Two types of enzymes vere used and the NOVO enzyme (Lipozyme TM) appears to be best suited as it is currently commercially available. Other biotechnology firms are also nov supplying lipase for the reaction and the most economical source should be searched out by any company wishing to employ this new technology. It should be noted that patents on this technology do exist (e.g. Unilever Ltd in England), and these should be examined prior to using these techniques.

CONCLUSIONS AND IMPACT

Biotechnology will influence several areas of the food processing industry. One area of impact is the production of enzymes that have potential for use in the food processing industry. This study evaluated the scientific merits of using one of these enzymes (lipase) to produce a Canola based margarine stock. The lipase was used to enzymatically interesterify Canola oil and fully

saturated Rape seed oil. The results clearly indicate that these new oil processing methods work with Canola oil and that a low trans-fatty acid, all Canola, margarine stock can be produced.

The principal investigator for this study spent four months working with a large oil processing company (Canada Packers Ltd. in Toronto, Ontario) attempting to commercialize this process. While the visit was not a component of this study, this industrial exposure has influenced the conclusions drawn. The scientific merits of the process were revalidated in the industrial laboratories, and the results suggest that investigations related to engineering scale-up should be implemented. Consideration should also be given to the economic implications of this new processing methodology.

TABLE 1

Fatty Acid (carbon number: double bond)	Canola Oil Mole %	Triacylglycerols from Interesterified Samples	
		Canola Oil:Stearic Acid (2:1) mole %	Canola Oil:Lauric Acid (2:1) mole %
12:0	--	--	25.50
14:0	0.52	--	0.70
16:0	4.00	2.73	4.30
18:0	2.22	25.53	3.02
18:1	65.10	55.30	40.42
18:2	18.72	11.22	15.72
18:3	6.17	3.08	7.44
20:0	1.19	0.91	1.43
22:0	0.40	--	0.82
22:1	0.70	1.22	0.63

TABLE 2

Free Fatty Acids Produced by Enzymatic or Chemical Interesterification of Canola Oil:HEAR Stearine Mixtures

Ratio of Canola Oil to HEAR Stearine	% of Free Fatty Acid as Oleic Acid			
	Mixture	Interesterified By		
		Pancreatic Lipase	NOVO "lipozyme"	Chemical
60:40	0.04	1.04	2.30	0.04
70:30	0.04	2.20	2.19	0.06
80:20	0.03	1.98	2.23	0.11
90:10	0.03	1.36	2.53	0.17

TABLE 3

Fatty Acid Composition of Fully Hydrogenated High Erueic Acid Rape Seed Oil (HEAR Stearine) and a Mixture of Canola Oil and HEAR Stearine (60:40).

Fatty Acid	Weight %	
	HEAR Stearine	Mixture of Canola Oil + Stearine (60:40)
16:0	4.12	4.76
18:0	50.12	20.26
18:1	---	39.06
18:2	---	12.01
18:3	---	4.27
20:0	12.34	5.04
22:0	33.42	13.73
24:0	---	0.87

FIGURE CAPTIONS

Figure 1. HPLC profiles of Canola triacylglycerols. A. Five percent lauric acid enzymatically interesterified with Canola. B. Five percent lauric acid mixed with Canola oil.

Figure 2. HPLC profiles of Canola triacylglycerols. A. Stearic acid enzymatically interesterified with Canola oil (33% stearic acid). B. Lauric acid enzymatically interesterified with Canola oil (33% lauric acid). C. Canola oil.

Figure 3. Relationship between the initial moisture in the reaction system and the free fatty acid level produced during enzymatic interesterification.

Figure 4. HPLC profile of the triacylglycerols in Canola containing 40% HEAR Stearine. A. 72 h at 60°C, 0.125% moisture in the reaction mixture. B. 24 h at 60°C, 0.4% moisture in the reaction system. C. Uninteresterified mixture.

Figure 5. HPLC profile of the triacylglycerols in: A. Enzymatically interesterified Canola oil and HEAR Stearine (40%). B. Chemically interesterified Canola oil and HEAR Stearine.

Figure 6. HPLC profiles of enzymatically interesterified Canola and HEAR Stearine. A. 10% HEAR Stearine, B. 20% HEAR Stearine, C. 30% HEAR Stearine, D. 40% HEAR Stearine, E. 50% HEAR Stearine

Figure 7. The softening points of enzymatically interesterified samples, chemically interesterified samples and mixtures of Canola oil and various levels of HEAR Stearine.

Figure 8. The melting points of enzymatically interesterified samples, chemically interesterified samples and mixtures of Canola oil and various levels of HEAR Stearine.

Figure 9. The cloud points of enzymatically interesterified samples, chemically interesterified samples and mixtures of Canola oil and various levels of HEAR Stearine.

Figure 10. Viscosity vs temperature for Canola oil mixtures, enzymatically interesterified samples, and chemically interesterified samples with 4 different levels of HEAR Stearine.

Figure 11. The solid fat index vs temperature for enzymatically interesterified samples, chemically interesterified samples, and mixtures of Canola oil containing: A. 40% HEAR Stearine, B. 30% HEAR Stearine, C. 20% HEAR Stearine, and D. 10% HEAR Stearine.

Figure 12. The increase in peroxide value with time for a enzymatically interesterified sample, a chemically interesterified sample and a mixture of Canola oil and HEAR Stearine (40% HEAR Stearine). The samples were stored at room temperature (23-25^{o}C) in screv cap jars.

FIGURE 1

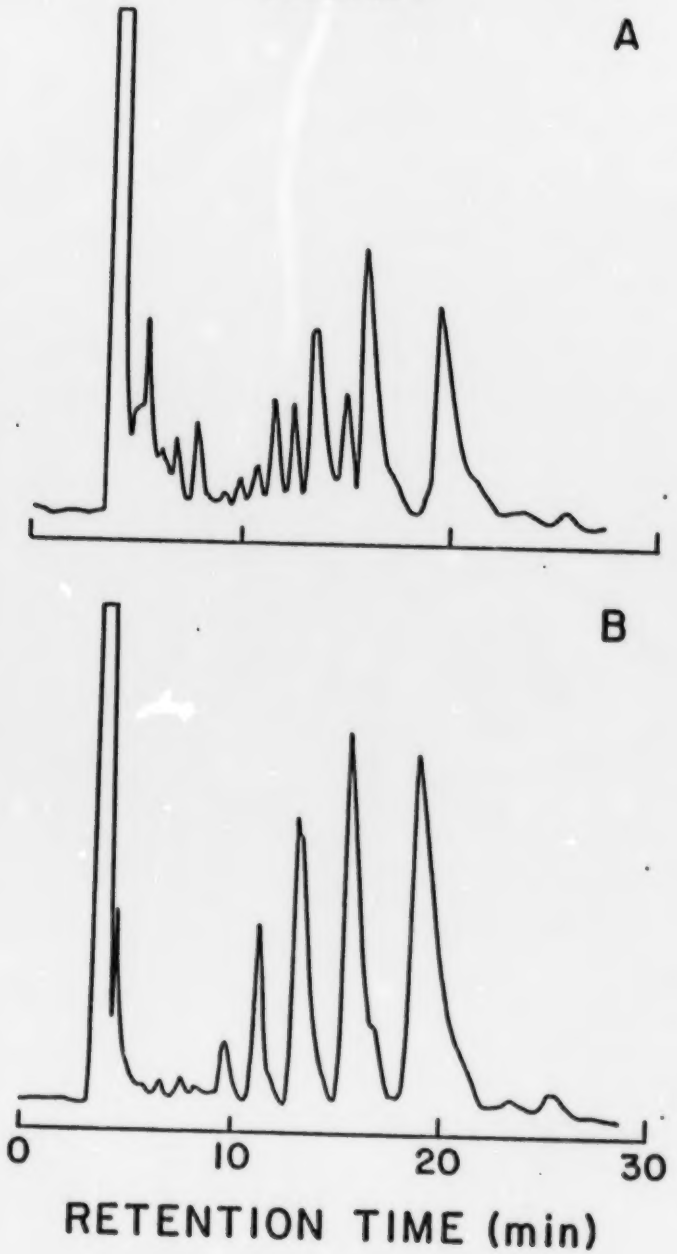


FIGURE 2

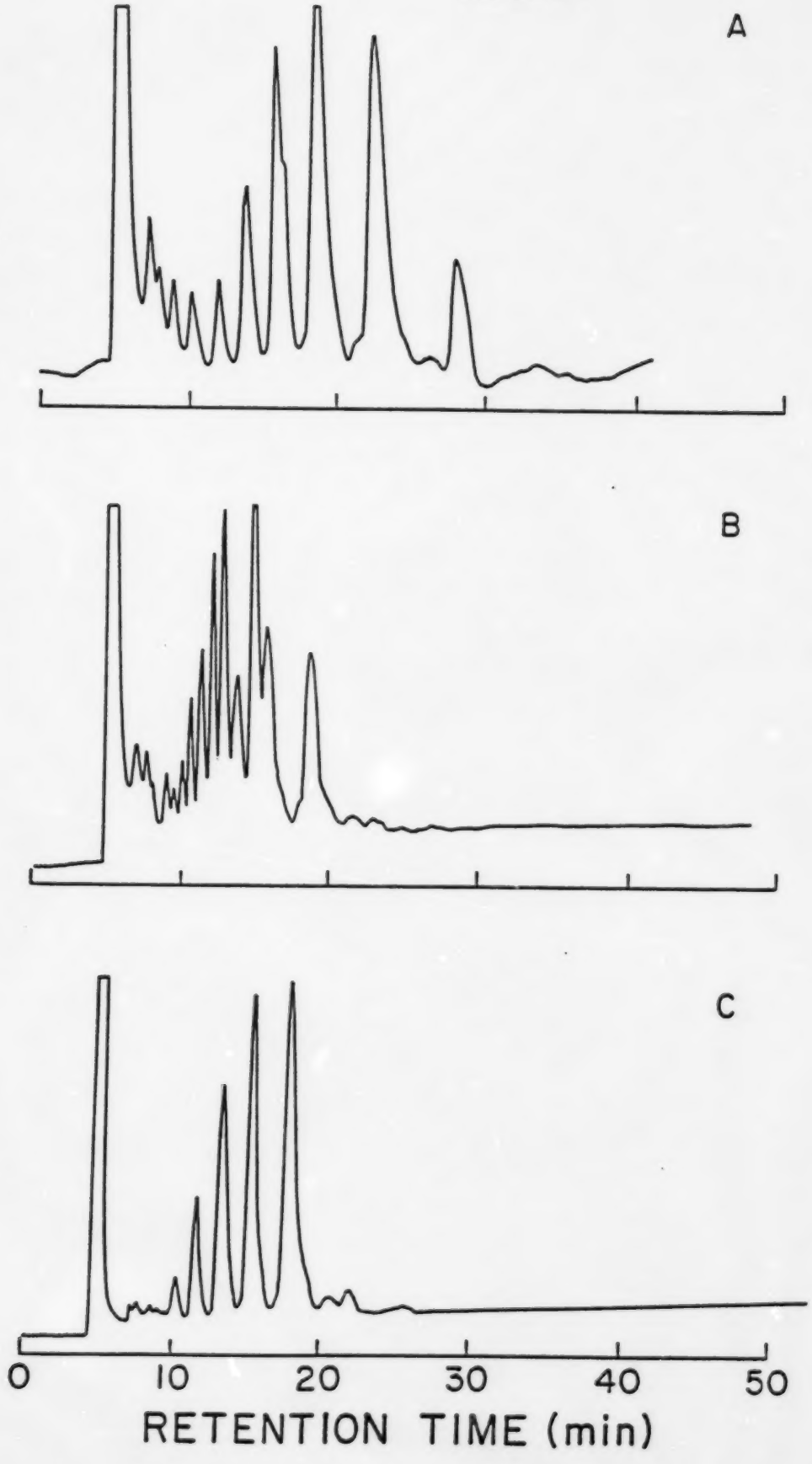


FIGURE 3

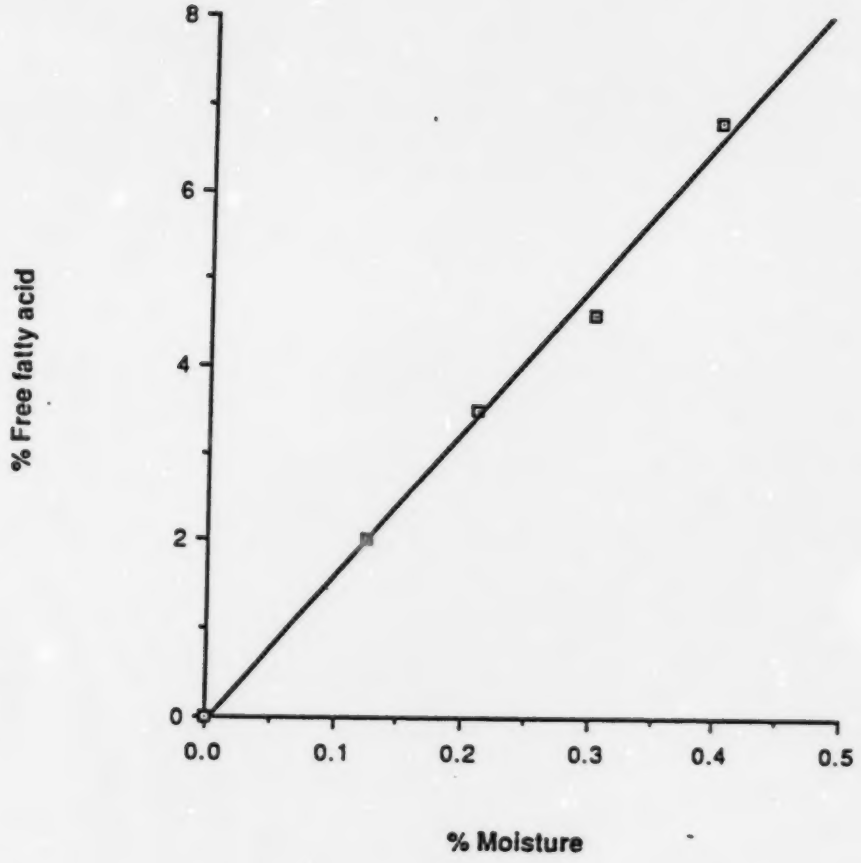
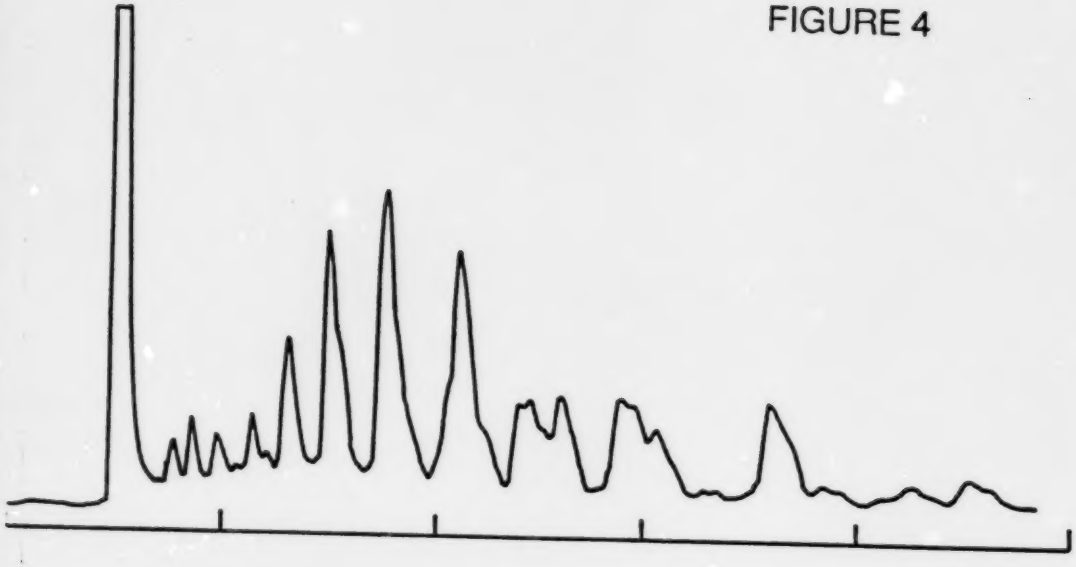


FIGURE 4

A



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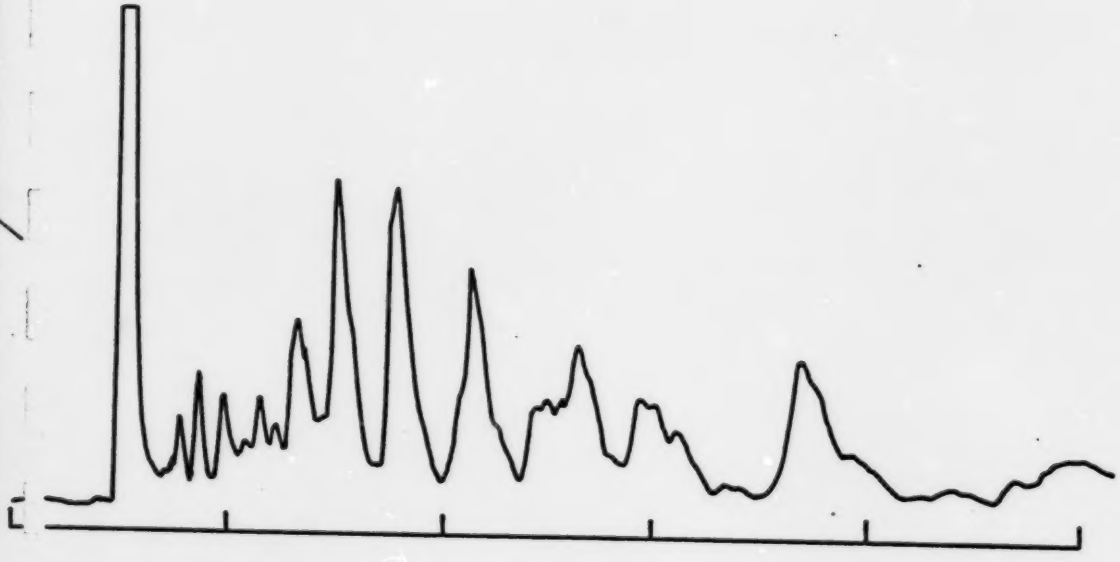


FIGURE 5

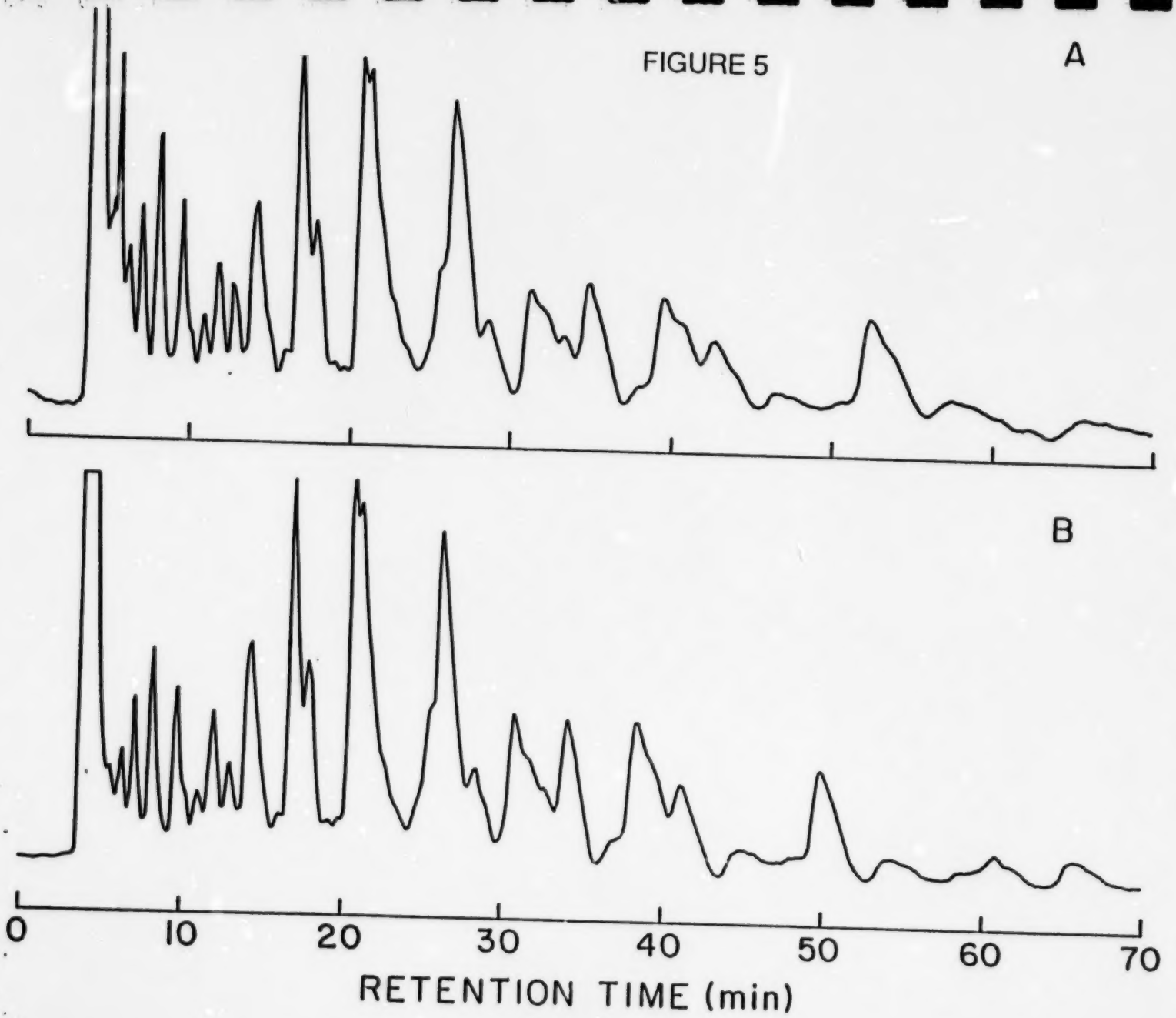


FIGURE 6

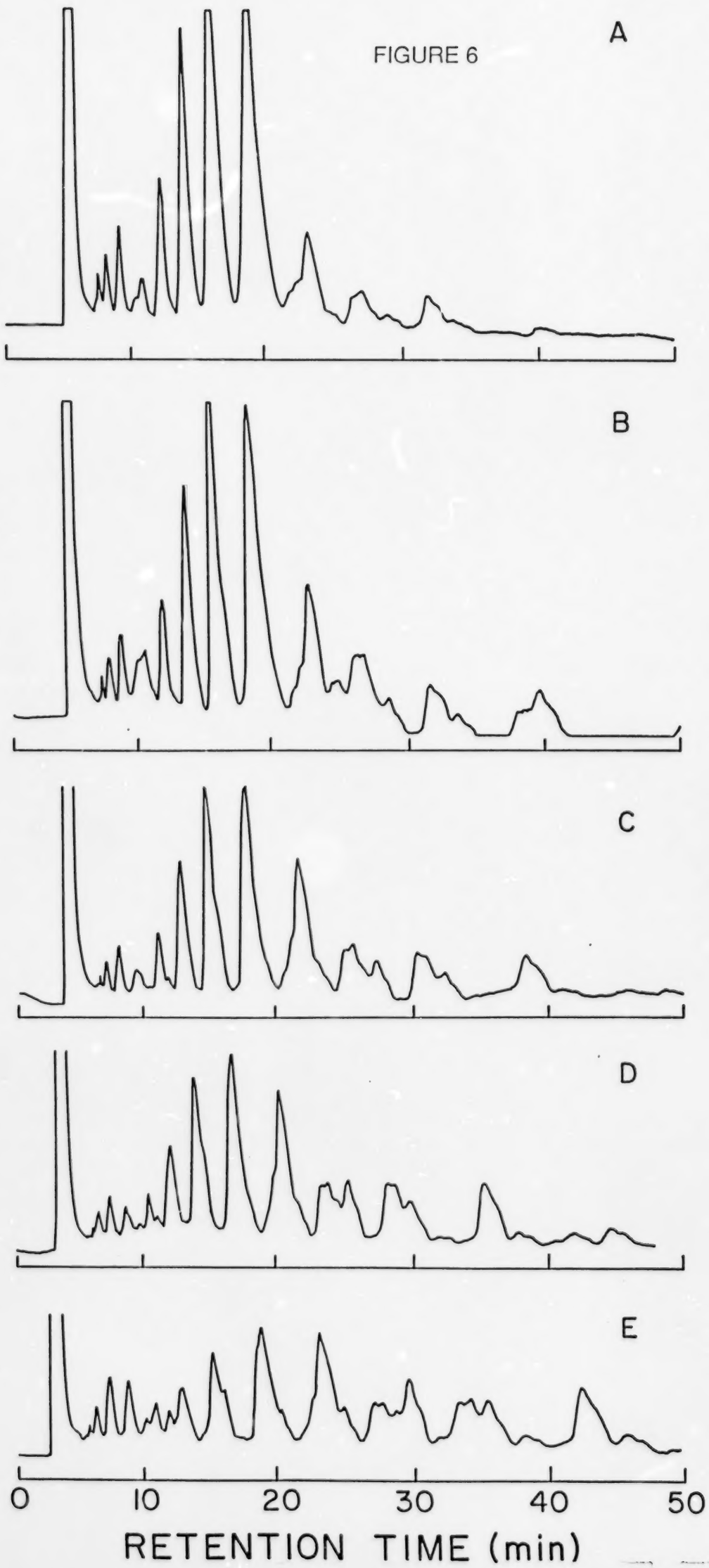


FIGURE 7

SOFTENING POINTS FOR

CANOLA:HEAR STEARINE COMBINATIONS

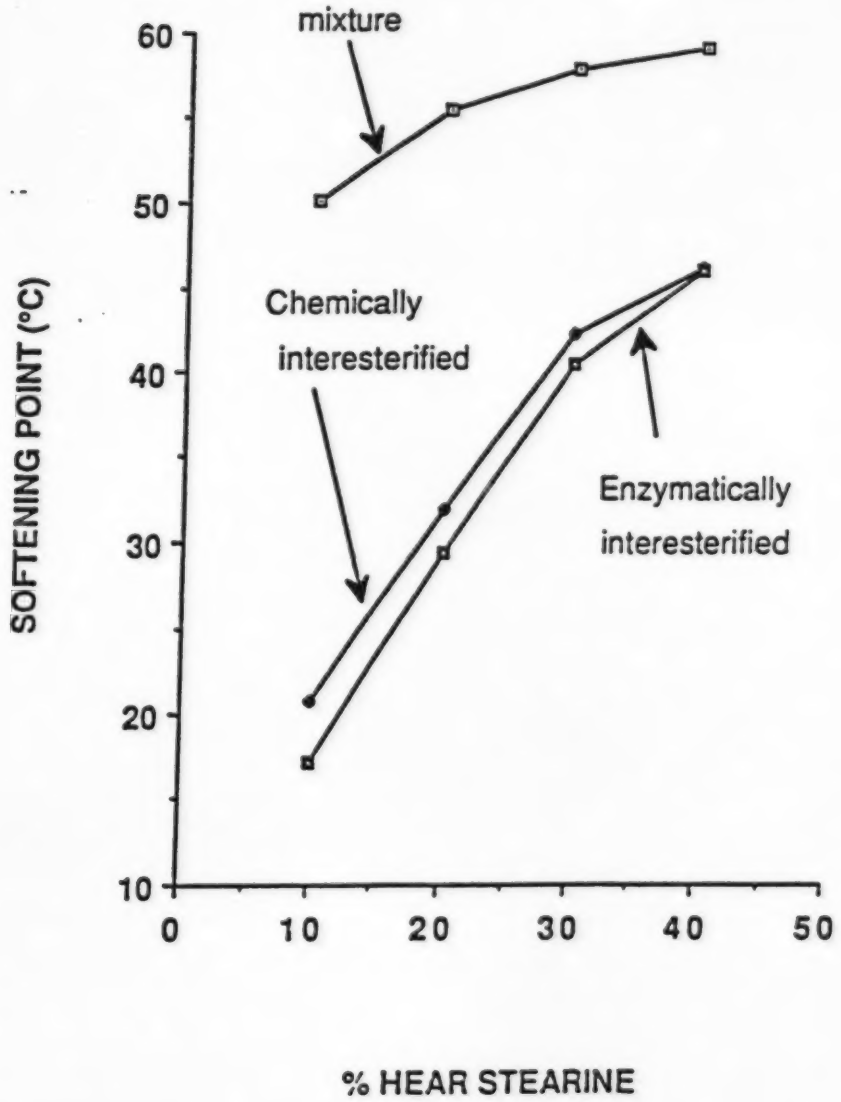


FIGURE 8

CAPILLARY MELTING POINTS

CANOLA:HEAR STEARINE COMBINATIONS

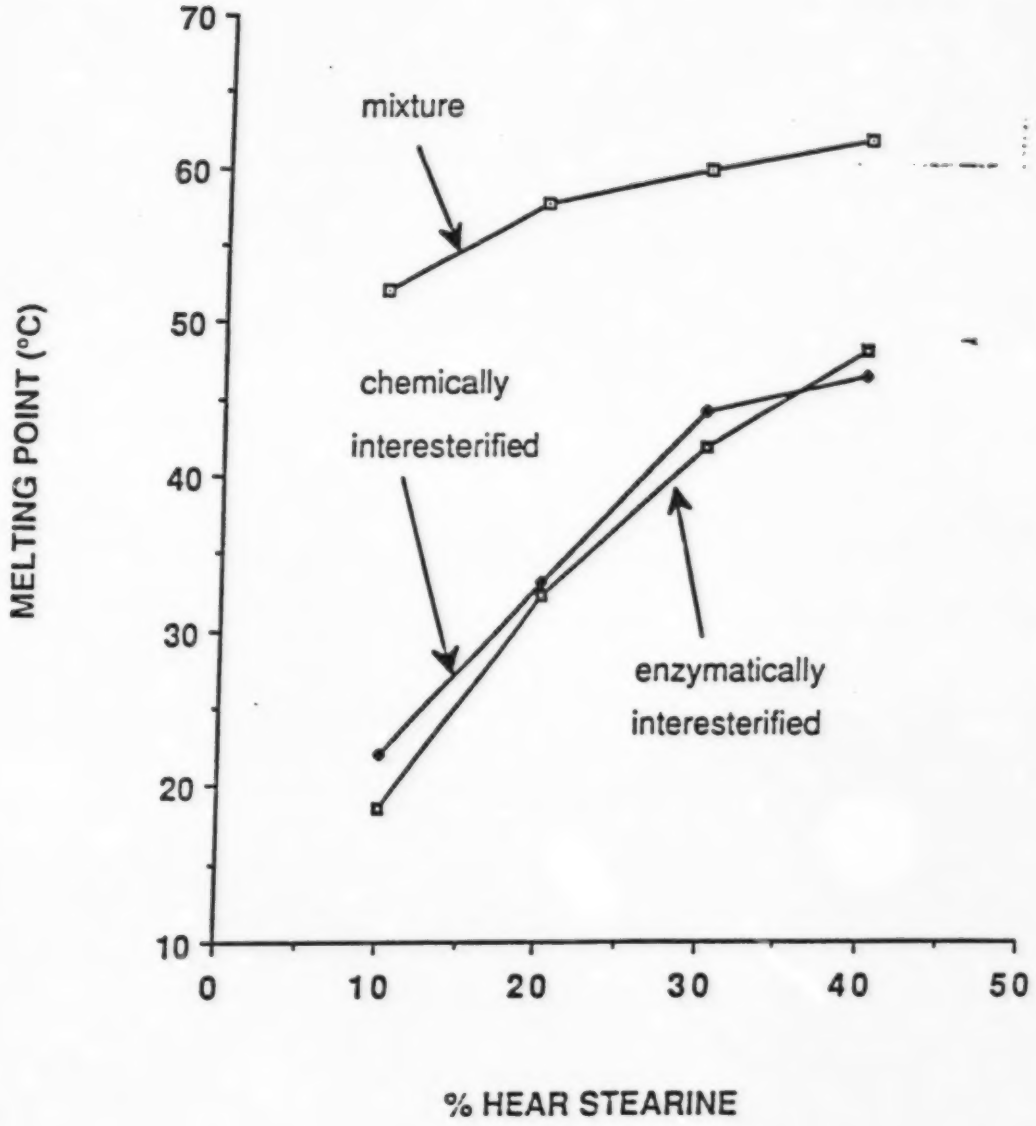


FIGURE 9

CLOUD POINTS FOR

CANOLA:HEAR STEARINE COMBINATIONS

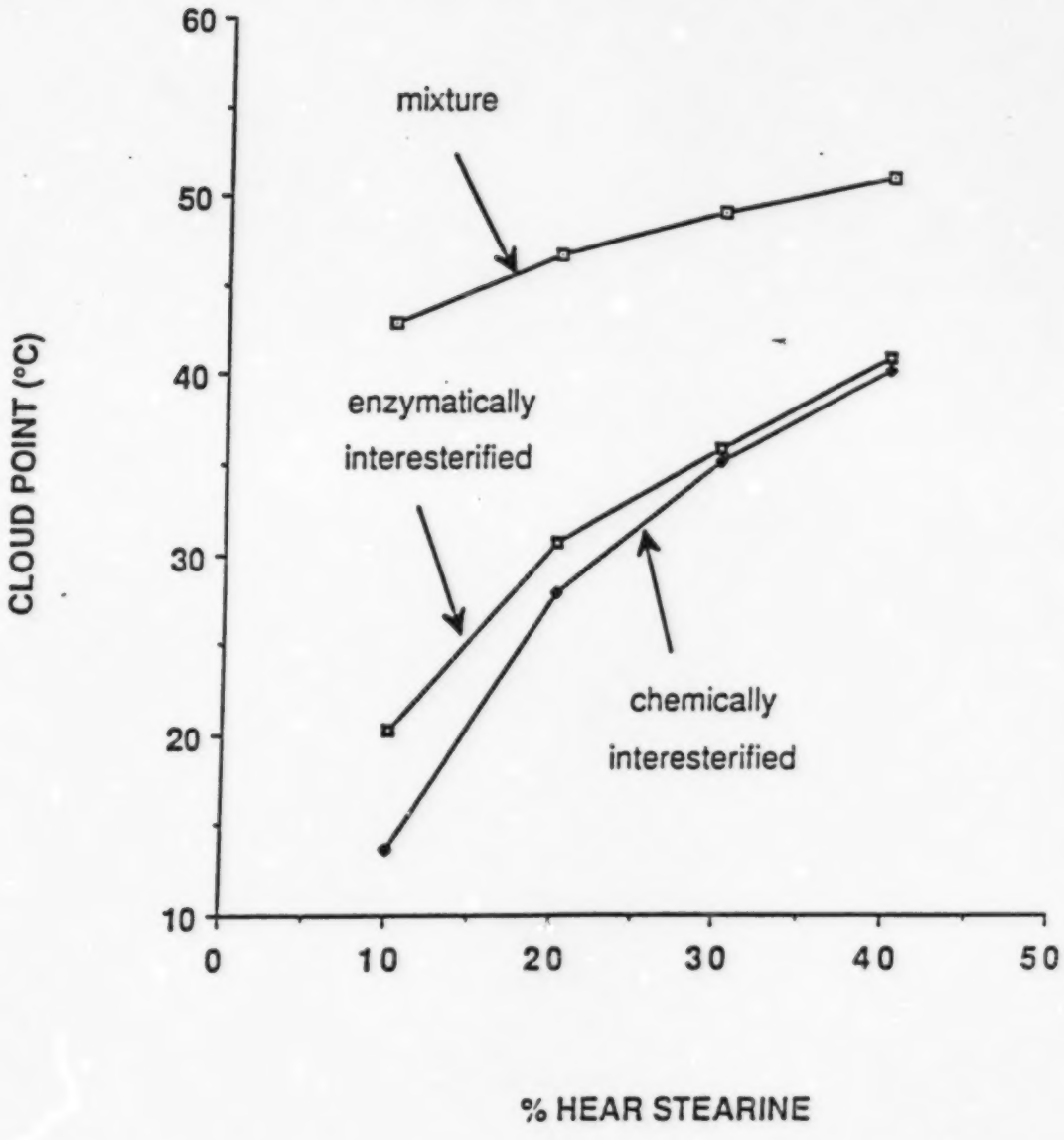
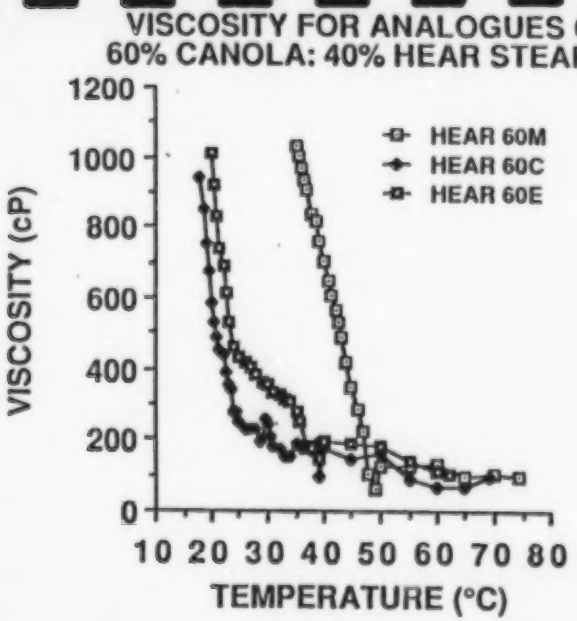
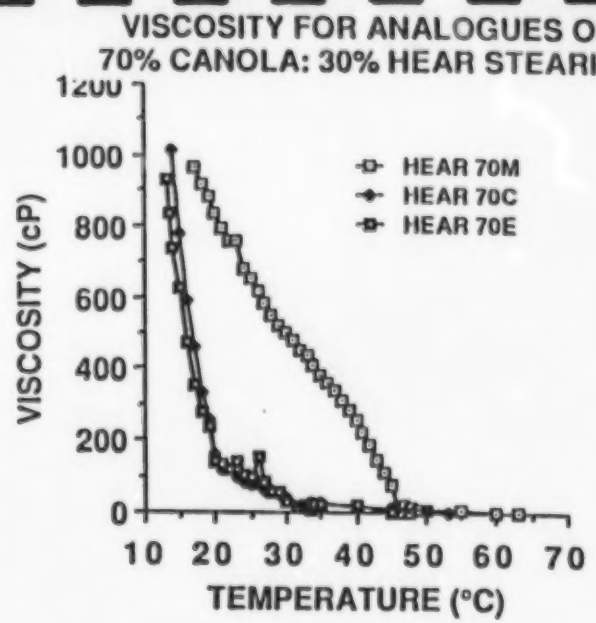
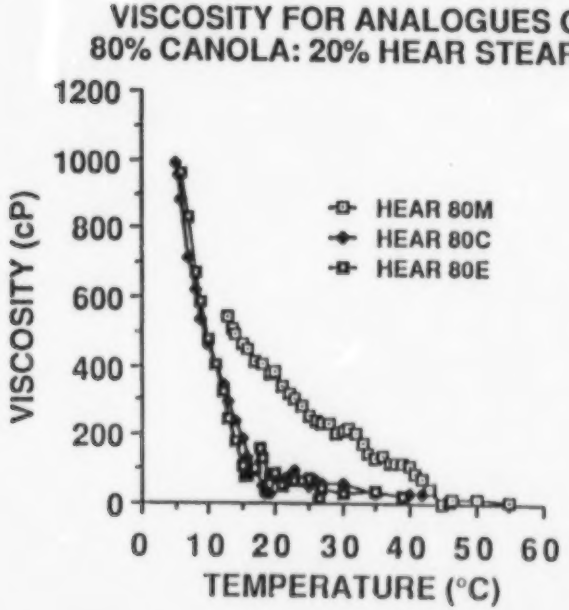


FIGURE 10


VISCOSITY FOR ANALOGUES OF
60% CANOLA: 40% HEAR STEARINE
HEAR 60M
HEAR 60C
HEAR 60E
VISCOSITY (cP)
TEMPERATURE (°C)


VISCOSITY FOR ANALOGUES OF
70% CANOLA: 30% HEAR STEARINE
HEAR 70M
HEAR 70C
HEAR 70E
VISCOSITY (cP)
TEMPERATURE (°C)


VISCOSITY FOR ANALOGUES OF
80% CANOLA: 20% HEAR STEARINE
HEAR 80M
HEAR 80C
HEAR 80E
VISCOSITY (cP)
TEMPERATURE (°C)

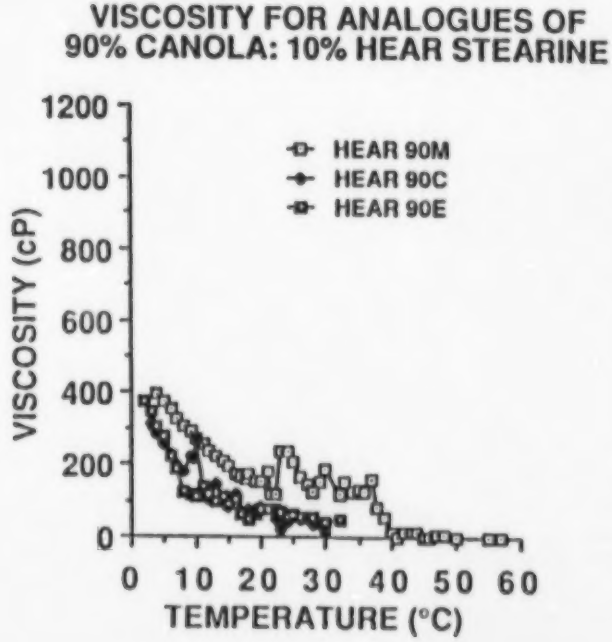
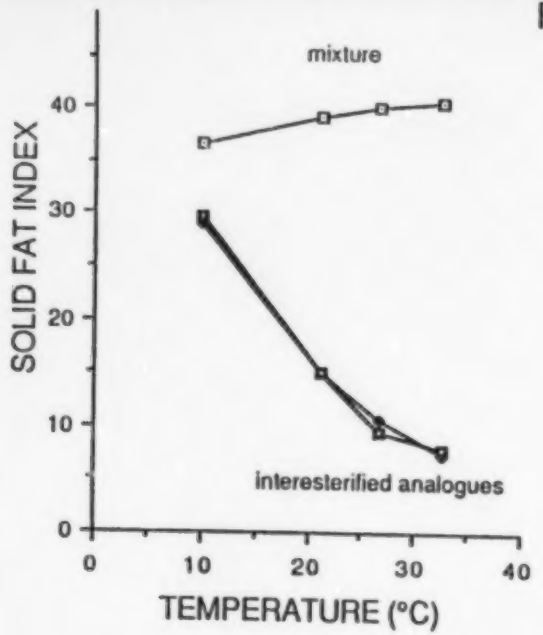
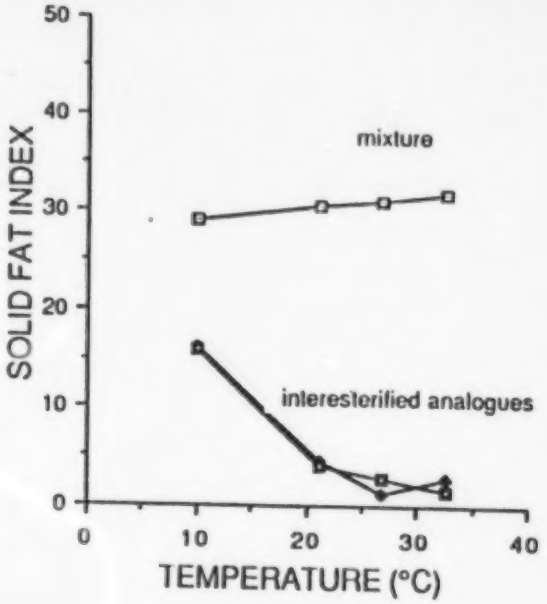

VISCOSITY FOR ANALOGUES OF
90% CANOLA: 10% HEAR STEARINE
HEAR 90M
HEAR 90C
HEAR 90E
VISCOSITY (cP)
TEMPERATURE (°C)

FIGURE 11



A)



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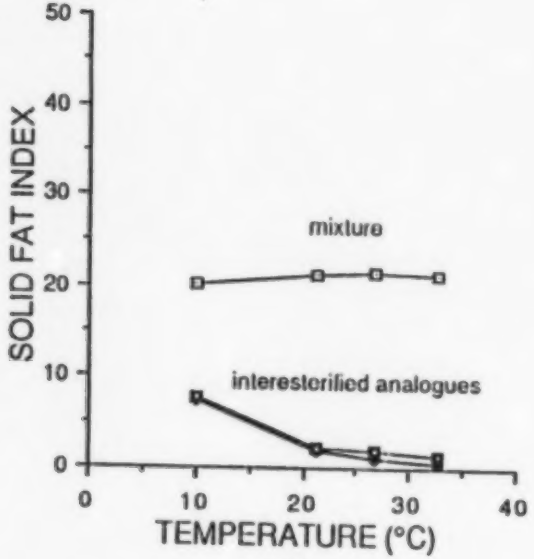

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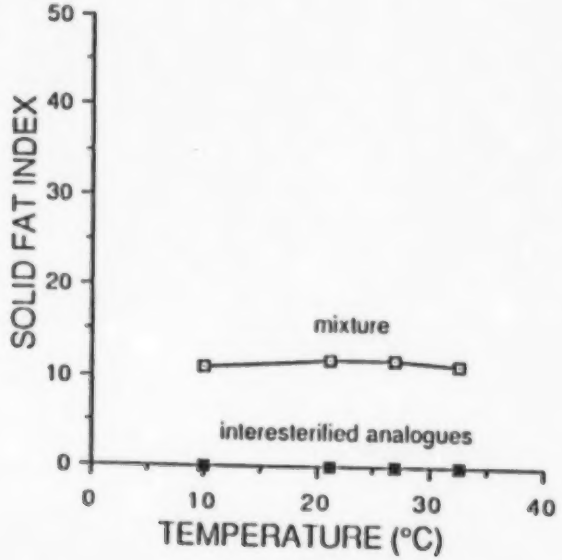

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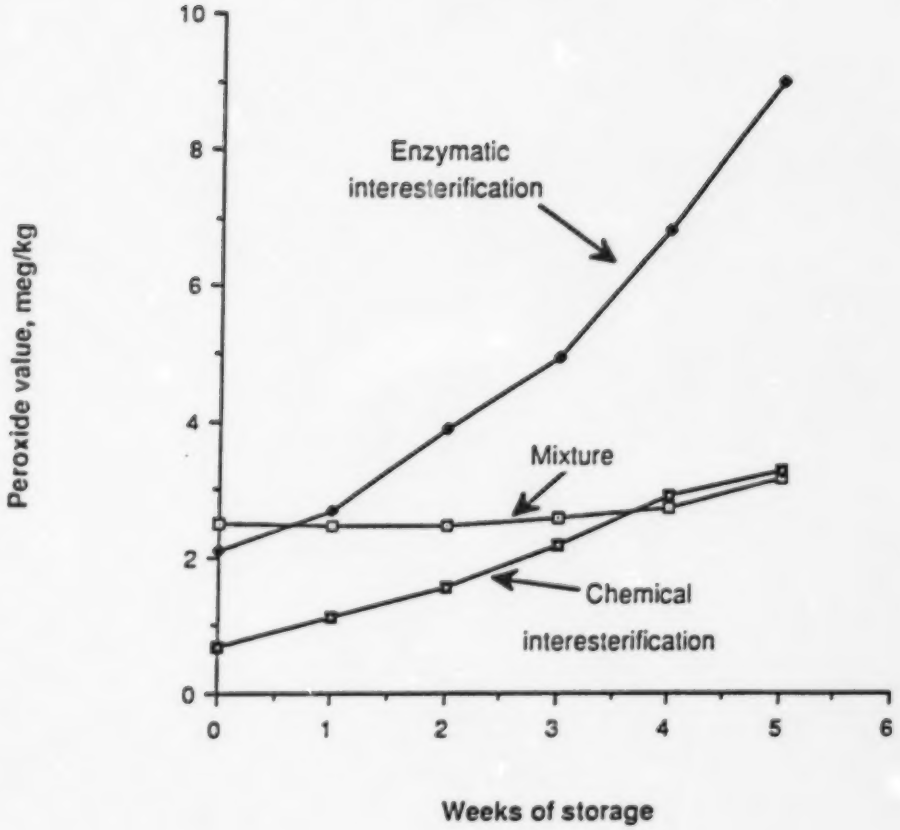
FIGURE 12


10
8
6
4
2
0
0
1
2
3
4
5
6
Peroxide value, meq/kg
Weeks of storage
Enzymatic
interesterification
Mixture
Chemical
interesterification